(g) *Calculations*—(1) Calculate the micrograms of cefoperazone per milligram of sample as follows:

$$\text{Micrograms of cefoperazone per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the cefoperazone sample peak (at a retention time equal to that observed for the standard);

A_s=Area of the cefoperazone working standard peak;

P_s=Cefoperazone activity in the cefoperazone working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m= Percent moisture content of the sample.

(2) Calculate the cefoperazone content of the vial as follows:

$$\text{Milligrams of cefoperazone per vial} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the cefoperazone sample peak (at a retention time equal to that observed for the standard);

A_s=Area of the cefoperazone working standard peak;

P_s=Cefoperazone activity in the cefoperazone working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

[48 FR 789, Jan. 7, 1983; 48 FR 7439, Feb. 22, 1983; 48 FR 28250, June 21, 1983]

§ 436.339 High-pressure liquid chromatographic assay for bleomycin fractions.

(a) *Equipment.* A high-pressure liquid chromatograph equipped with:

(1) Two solvent pumps;

(2) A solvent programmer;

(3) A low dead volume cell 8 to 20 microliters;

(4) A light path length of 1 centimeter;

(5) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(6) A suitable recorder;

(7) A suitable integrator; and

(8) A suitable-sized column approximately 25 centimeters in length having an inside diameter of 4.6 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter, USP XX.

(b) *Reagents*—(1) *0.005M 1-pentanesulfonic acid in 0.5 percent acetic acid adjusted to pH 4.3 with concentrated ammonium hydroxide.* Filter and degas before using.

(2) *Methanol, spectrophotometric grade.* Filter and degas before using.

(3) *Mobile phase.* Adjust the solvent programmer for linear gradient development starting with a mixture of 0.005*M* 1-pentanesulfonic acid:methanol (9:1) and ending with a mixture of 0.005*M* 1-pentanesulfonic acid:methanol (6:4) in 1 hour at a flow rate of 1.2 milliliters per minute. Minor flow rate and gradient changes can be made as necessary depending on column and instrument conditions. Disodium ethylenediaminetetraacetic acid USP at a concentration of 0.005*M* may be added to the mobile phase if necessary for satisfactory performance.

(c) *Preparation of sample solution.* Reconstitute the vial with 6 milliliters of deaerated water.

(d) *Procedure.* Using the equipment and reagents listed in paragraphs (a) and (b) of this section, start pumping the mobile solvent at the initial conditions. Inject 10 microliters of the sample solution into the chromatograph and begin the linear gradient pumping program. After the final mobile phase conditions are reached (1 hour) continue to pump the solvent mixture for an additional 20 minutes or until the demethylbleomycin A_2 is eluted. The elution order is void volume, bleomycinic acid, bleomycin A_2, bleomycin A_5, bleomycin B_2, bleomycin B_4, and demethylbleomycin A_2.

(e) *Calculations.* Calculate the percentage of each bleomycin by comparing its peak area contribution to that of the total response of all the bleomycins.

[48 FR 51912, Nov. 15, 1983]

§ 436.340 High-pressure liquid chromatographic assay for tetracycline hydrochloride content and 4-epitetracycline hydrochloride content.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;